Chemokines and Chemokine Receptors: Potential Therapeutic Targets in Multiple Sclerosis

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Abstract: Multiple sclerosis (MS) is a common inflammatory and demyelinating disease of the central nervous system (CNS), which causes progressive neurological disability. The disease is characterised pathologically by destruction of the myelin sheaths, which surround nerve fibres in the CNS. It is believed that this tissue damage in the brain and spinal cord of MS patients is caused by an inflammatory response that is initiated when autoreactive T cells, specific for myelin antigens, cross the blood-brain barrier and detect their antigen within the CNS. As a result, most therapies to date have been immunosuppressive and/or anti-inflammatory in nature, targeting the processes involved in activation and migration of leukocytes and promotion of the immune response. Over the last decade, a family of chemotactic cytokines called chemokines, have been found to be involved in the trafficking of leukocytes in both the normal and pathological states. The expression of these chemokines and their receptors is increased during the acute phase of MS and also in the animal model of MS, experimental autoimmune encephalomyelitis (EAE). As a result, these chemokines have become an emerging focus for research into novel therapeutics for EAE and ultimately MS. This review will briefly describe the structure and function of chemokines and their receptors, before discussing the latest advances in developing pharmacological agents to block the effects of chemokines involved in promoting the inflammatory response in EAE and MS.

Keywords: Chemokine, chemokine receptor, chemotaxis, multiple sclerosis, inflammation, therapy.

INTRODUCTION TO CHEMOKINES AND THEIR RECEPTORS

Chemokines are a family of small 8-10kDa proteins produced by a variety of cells either constitutively or in response to injury or infection in the host tissue [1]. Chemokines are involved in many biological processes including angiogenesis, growth regulation, Human Immunodeficiency Virus (HIV) infection, hematopoiesis, and organogenesis. However, the best understood functions of these molecules are firstly the recruitment of inflammatory cells/leukocytes into injured or affected tissue during host defense, either through direct chemoattraction or by activating cell surface integrins to aid in binding to adhesion receptors on vascular endothelial cells (inflammatory chemokines) [2,3], and, secondly, the maintenance of lymphocyte homeostasis by constitutively expressed chemokines directing lymphocyte homing and maturation [4,5].

The human chemokine system is a rapidly growing field of research. To date, there have been more than 50 chemokines described, and this number continues to grow. Recently, the nomenclature of the chemokine system has been revised by the IUIS/WHO Subcommittee on Chemokine Nomenclature [6]. Table 1 shows the major chemokines reported in humans to date and details the changes in chemokine nomenclature. Table 1 also describes the cellular source and roles of chemokines. This table is not exhaustive and contains information relevant to this review. For further information, the reader is directed to the Online Cytokine Reference at www.academicpress.com/companions/0122526708.

The chemokine family is divided into four subfamilies, based on the structure of NH_2-terminal cysteine motifs: C, CC, CXC, CX3C. The C or α family has only one cysteine residue at the NH_2-terminal. The CC or β family has the first two cysteine residues adjacent. The CXC or family has one amino acid separating the first two conserved cysteines, whereas the CX3C or δ family has three amino acids between the first and second cysteine residues in the chemokine domain.

The CXC family can be divided further into two groups based on the presence or absence of a sequence of amino acids (glutamic acid-leucine-arginine) called the ELR motif that precedes the first cysteine residue near the amino terminus. Members of this ELR-containing group appear to have a functional uniformity in that they activate and are chemotactic for neutrophils, and they are also angiogenic, but appear to have little effect on mononuclear cells. The CXC chemokines which lack the ELR motif are chemotactic for lymphocytes and monocytes but are poor chemoattractants for neutrophils [7].

In order for chemokines to elicit their biological effects they must interact with their target receptor on resident or infiltrating cells. Chemokine receptors belong to the G protein coupled receptor (GPCR) superfamily. These receptors contain an extracellular NH_2 terminus, seven-transmembrane domains and a cytoplasmic COOH-terminus. Associated with the receptor are heterotrimeric G proteins consisting of α and $\beta\gamma$ subunits (Fig. (1)).

Binding of the chemokine with its target receptor initiates an intracellular signal transduction cascade which begins with a conformational change in the receptor, causing dissociation of the G protein into its α and $\beta\gamma$ subunits. These subunits, particularly the $\beta\gamma$ subunits, activate a chain of cellular enzymes, via a phospholipase C and a phosphatidylinositol-3-OH kinase pathway. This leads to inositol 1,4,5-triphosphate and diacylglycerol production, an increase in the intracellular Ca^{++} ions and activation of other protein kinases, resulting in upregulation of adhesion proteins and integrins. The modulation of actin-dependent cellular processes leads to chemotaxis and transmigration. Leukocytes migrate along a gradient of increasing concentration of the chemokine to the target site. Other cellular processes are also elicited as a result of this signal transduction cascade, including an increase in respiratory burst, phagocytosis and degranulation, depending on the type of cell involved [3,4,8]. Table 2 details the chemokines receptors that have been described to date. As for Table 1, Table 2 is not exhaustive and the reader is directed to the aforementioned online cytokine reference source.

As can be seen from Tables 1 and 2, there is an apparent redundancy in the chemokine/chemokine receptor system. Chemokine receptors bind chemokines only in one of the four chemokine families; however, within that family, one receptor may bind several chemokines. (The revised nomenclature aims to increase the ease of understanding of which subfamily of chemokine receptors interact with which subfamily of chemokines by adding an R for "receptor" to the chemokine subfamily name.) A similar situation is present for the chemokines, in that one chemokine may be able to bind with high affinity to more than one chemokine receptor. The reason for this redundancy is still unclear but it is thought that if one chemokine receptor/ chemokine is negated then another will take over its functions [9].

Chemokine receptor expression can be constitutive, or based on the activation state of the cell, or induced by other inflammatory agents. Chemokine receptor expression is also determined by the stage of cell development, e.g. T lymphocytes express different receptors depending on the state of maturation, activation, and whether they are Th1 or Th2 polarised. This is also true for other cells like dendritic cells and B cells. The receptor expression dictates their responsiveness to various chemokines which impacts on the migration, homing and functional properties of the cells. Furthermore, on the basis of receptor expression, information is emerging detailing further subsets existing within immune cell classes.

As chemokines are such an important participant in homeostasis and in the normal immune response, it is reasonable to assume that when the immune response is inappropriately targeted toward normal tissue, as is the case in autoimmunity, chemokines will be involved.

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Table 1. Chemokines

CHEMOKINE	ORIGINAL NAMES	CELLULAR SOURCE*	ROLE	RECEPTORS
CXC (α family)				
CXCL1	GRO-α / KC	MΦ, N, EC, L	Chemotaxis/activation of granulocytes, monocytes, lymphocytes; acute inflammation	CXCR2
CXCL2	GRO-β / MIP-2	MΦ, N, EC, L	Chemotaxis/activation of granulocytes, monocytes, lymphocytes; acute inflammation; angiogenesis	CXCR2
CXCL3	GRO-γ	MΦ, N, EC, L	Chemotaxis/activation of granulocytes, monocytes, lymphocytes; acute inflammation	CXCR2
CXCL4	PF4	Mega	Inhibits angiogenesis	Unknown
CXCL5	ENA-78 / LIX	M, P	Chemoattractant and activator of neutrophils	CXCR2
CXCL6	GCP-2	M, F, Ost	Chemoattractant for neutrophils	CXCR1, CXCR2
CXCL7	NAP-2	P, Mega, T, N, M	Activates neutrophil effector function	CXCR2
CXCL8	Interleukin-8	M, L, G, F, EC	Chemotaxis and activation of neutrophils; induces angiogenesis; inflammation	CXCR1, CXCR2
CXCL9	Mig	MΦ, EC, F, Micro, A, L, N	Chemotaxis of activated T and natural killer cells; inflammation	CXCR3
CXCL10	IP-10	M, A, Micro, EC, N	Chemotactic for activated T cells and natural killer cells; inhibits angiogenesis; inflammation	CXCR3
CXCL11	I-TAC	A, MΦ, N	T cell chemotaxis; inflammation	CXCR3
CXCL12	SDF-1α/β	Stromal cells	Attracts T cells; B cells; haematopoietic progenitors	CXCR4
CXCL13	BCA-1	Secondary lymphoid organs	B cell chemotaxis; homeostasis	CXCR5
CXCL14	BRAK	Breast, kidney	Host tumour interactions; homeostasis	Unknown
CXCL16		Spleen, DC, Mono, B	T cell trafficking; inflammation	CXCR6
CC (β Family)				
CCL1	I-309 / TCA-3	T, M	Chemotactic for monocytes and Th2 T cells; inflammation	CCR8
CCL2	MCP-1	M, MΦ , N, F, EC, A	Chemotactic for Monocytes, T cells, natural killer cells; induce histamine release from basophils; inflammation	CCR2
CCL3	MIP-1α	MΦ, L, N, EC, F, Mono	Attractant for Monocytes, macrophages, neutrophils, eosinophils,dendritic cells, natural killer, subsets of T cells; inflammation	CCR1, CCR5
CCL3L1	LD78β	T, B, Tu	Promotion of lymphocytes; adherence of monocytes to endothelial cells	CCR1, CCR5
CCL4	MIP-1β	MΦ; L; N; F; MC	Chemotactic for Monocytes, dendritic cells, natural killer, T cells; inflammation	CCR5
CCL5	RANTES	MΦ, EC, Epi, T, Me, E	Chemotactic for Monocytes, dendritic cells, T cells, natural killer cells, eosinophils, basophils; inflammation	CCR1, CCR3, CCR5
CCL7	MCP-3	P, M, F, A	Chemotactic for Monocytes, T cells, natural killer cells; induce histamine release from basophils, eosinophils; inflammation	CCR1, CCR2, CCR3
CCL8	MCP-2	F, N, A	Chemotactic for Monocytes, T cells, natural killer cells, eosinophils; induce histamine release from basophils ; inflammation	CCR3, CCR5
CCL11	Eotaxin-1	EC, Epi, E, Mast, MΦ, F	Attracts Eosinophils, T cells, basophils; inflammation	CCR3
CCL13	MCP-4	EC, F, M	Chemotactic for monocytes, T cells, natural killer cells, eosinophils; induce histamine release from basophils; inflammation	CCR2, CCR3

(Table 1) contd.....

CHEMOKINE	ORIGINAL NAMES	CELLULAR SOURCE*	ROLE	RECEPTORS
CCL14	HCC-1	Skeletal and heart muscle; spleen; liver; colon	Chemotaxis, Ca^{++} changes and enzyme release in monocytes	CCR1, CCR5
CCL15	MIP-1δ / HCC-2	T, B, NK, M, DC	Chemoattractant to lymphocytes, monocytes, neutrophils and eosinophils	CCR1, CCR3
CCL16	HCC-4	M, low levels in some T and NK	Chemoattractant for monocytes, T cells; cell adhesion	CCR1, CCR2
CCL17	TARC	DC	Attracts memory T cells, thymocyte migration; inflammation; homeostasis	CCR4
CCL18	PARC	Not known	Attractant for a subset of T cells; homeostasis	Unknown
CCL19	MIP-3β / ELC	DC; mRNA in MΦ and SM	Chemoattractant for T and B lymphocytes, dendritic cells, thymocytes; homeostasis	CCR7
CCL20	LARC / MIP-3α	L, thymus	Homing dendritic cells; attracts lymphocytes; inflammation; homeostasis	CCR6
CCL21	SLC	Lymphoid tissue	Migration of thymocytes, dendritic cells, lymphocytes; homeostasis	CCR7
CCL22	MDC	DC, MΦ, M, T, B	Attractant lymphocytes, monocytes, thymocytes, dendritic cells; inflammation; homeostasis	CCR4
CCL23	MPIF-1	DC, M	Attracts for lymphocytes, monocytes, neutrophils	CCR1
CCL24	Eotaxin-2	Not known	Chemotaxis of Th2 cells, eosinophils, basophils, dendritic cells; inflammation	CCR3
CCL25	TECK	Thymus; small intestine	Attracts thymocytes, macrophages, dendritic cells; homeostasis	CCR9
CCL26	Eotaxin-3	EC	Chemotactic for eosinophils and basophils; inflammation	CCR3
CCL27	CTACK / ALP/ IL C	K, placenta	Possible role in attracting memory T cells; homeostasis	CCR10
CCL28	MEC	Epi, L	T cell chemotaxis; inflammation; homeostasis	CCR3, CCR10
C (γ family)				
XCL1	Lymphotactin / SCM-1α	Activated T cells; mast cells; NK cells	Chemotaxis of T cells, natural killer cells	XCR1
XCL2	SCM-1β			XCR1
CX_3C (δ family)				
CX3CL1	Fractalkine	EC; DC; Neurons (mRNA); MΦ	Chemotaxis of T cells, monocytes, natural killer cells, microglia; assists in cell adhesion; inflammation	CX3CR1

*Abbreviations : A = astrocytes, B = B cells, DC = dendritic cells, E = eosinophils, EC = endothelial cells, Epi = epithelial cells, F = fibroblasts, G = granulocytes, K = keratinocytes, L = lymphocytes, M = monocytes, Me = mesangial cells, Mega = megakaryocytes, MΦ = macrophages, Micro = microglia, N = neutrophils, NK = natural killer cells, Ost = osteosarcoma cells, P = platelets, SM = smooth muscle cells, T = T lymphocytes, Tu = tumour cell lines

CHEMOKINES AND CHEMOKINE RECEPTORS IN MULTIPLE SCLEROSIS (MS)

An autoimmune disease in which chemokines have been implicated is multiple sclerosis (MS), an inflammatory demyelinating disease of the CNS. In MS, it is thought that host T cells become autoreactive to self myelin proteins and elicit an inflammatory response against myelin, resulting in inflammation, edema and demyelination. Typically, people with MS have lesions occurring in the white matter of the brain and spinal cord, although the gray matter can also be involved. Blood-brain barrier (BBB) disruption and increased levels of chemokines and chemokine receptors are a feature of this disease and are thought to contribute to the infiltration of inflammatory cells into the CNS and the formation of inflammatory and demyelinating lesions.

Whilst there is a moderate body of work published on chemokines in MS patients, most of the functional and invasive studies of the chemokine and chemokine receptor system have been done using the animal model of MS, experimental autoimmune encephalomyelitis (EAE). EAE can be induced in a wide variety of animals; however, the rat and the mouse are the most commonly used species. EAE is induced by injecting myelin proteins/peptides in adjuvant, or by injecting T cells specific for these peptides. This induces a disease that has histological features similar to those seen in MS. A concise review of EAE can be found in Gold *et al.* [10].

Pathological Pathways in MS and EAE

The proposed series of events involved in the inflammatory response in MS and EAE is that autoreactive T cells specific for myelin antigens are activated in the peripheral lymphoid organs, and circulate in the blood. As part of normal immune surveillance, activated T cells enter the perivascular space. If the myelin-specific T cells come into contact with

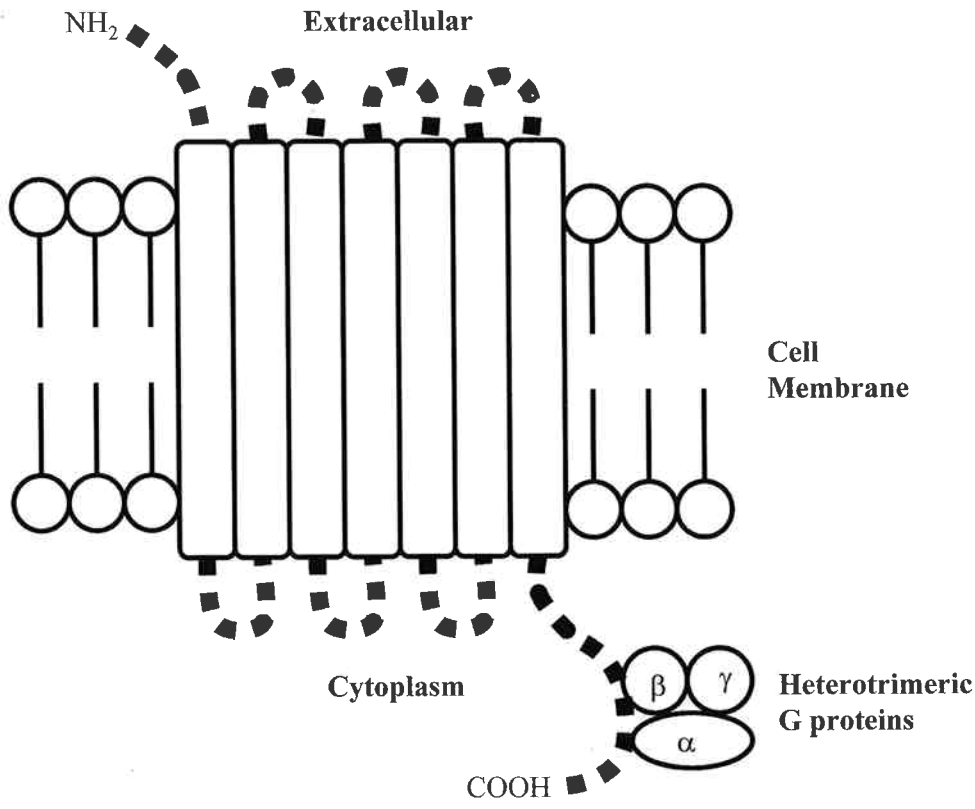

Fig. (1). Typical structure of a G protein coupled receptor.

their target antigens, they will remain in the perivascular space and produce proinflammatory cytokines such as tumour necrosis factor (TNF)-α and interferon (IFN)-γ, that stimulate the upregulation of chemokine expression in leukocytes and in resident glial cells of the CNS adjacent to the perivascular space, especially astrocytes and microglia. Expression of adhesion molecules is also upregulated on the vascular endothelium. [11,12]. Secondary leukocytes expressing chemokine receptors specific for the chemokines are attracted along a chemokine gradient from the blood across the BBB into the CNS. Once mononuclear cells adhere to endothelial cells, chemokine production is induced in the mononuclear cells [13], amplifying the response even more. The BBB loses functional integrity during large-scale leukocyte migration, possibly, in part, due to the matrix metalloproteinases (MMPs), which are found within inflammatory cells and glial cells and which can degrade basement membranes [14]. This degradation allows inflammatory factors and cells to be exposed to the CNS parenchyma [15]. The activated T cells, secondary leukocytes and resident cells in the CNS can then further stimulate the upregulation of cytokines, chemokines and their receptors, thus promoting the inflammatory response and inducing chemotaxis into the CNS parenchyma [16]. As a result, most MS lesions consist of T cells, other inflammatory cells, activated glia and activated endothelium.

Glial cells appear to be important in regulating the trafficking of cells and amplifying the response [17]. Glial cells not only produce chemokines [18,19] but also express chemokine receptors [20]. They also can migrate in response to chemokines as well as have their receptor expression upregulated by TNFα and IFNγ [21]. When astrocytes are stimulated with chemokines *in vitro*, they can release proinflammatory mediators and modulate the cell surface protein expression [22]. The chemokines expressed by glial cells could attract inflammatory cells, which in turn, express cytokines or chemokines that activate the glia and continue the cycle. Alternatively, chemokines produced by glial cells may act in autocrine fashion to upregulate their own expression. During lesion resolution reactive astrocytes no longer produce chemokines [23].

Role of Chemokines and Their Receptors in MS and EAE

Chemokines and their receptors have been reported to be involved at most steps in the pathogenic processes in MS and EAE. As the chemokine system orchestrates the migration and infiltration of inflammatory cells and thus the lesion composition in EAE and MS, it is important to understand their spatial and temporal expression. Spatial expression means the expression of chemokines or their receptors at certain anatomical sites during disease pathogenesis e.g. in the blood, at the BBB, in the cerebrospinal fluid (CSF), or in the brain parenchyma itself. Temporal expression is the expression of chemokines and their receptors at different stages of the disease process, for example in relapse or remission.

Differential Spatial Expression of Chemokines and Their Receptors in MS and EAE

Different chemokines and chemokine receptors are involved at different sites in the peripheral lymphoid organs and within the CNS during MS. Expression of these chemokines by various subpopulations of cells, both residential and infiltrating, can sometimes indicate the role that the particular chemokine or chemokine receptor may be playing in promoting the inflammatory response. The major findings in the cellular expression of chemokines and chemokine receptors in MS are summarised in Fig. (2).

The chemokines most commonly found to be involved in MS are CCL2, CCL3, CCL4, CCL5 and CXCL10. The anatomical sites, where these chemokines are expressed, help to indicate their putative function. The vascular endothelium expresses CCL2 [24] and CCL5 [19], which may indicate that these chemokines are involved in early lesion formation and assist in the extravasation of leukocytes from the blood. CCL2 upregulates adhesion molecule expression by human monocytes, which suggests that it is involved in transendothelial migration [53].

Following extravasation, inflammatory cells accumulate in the perivascular space before migrating into the surrounding CNS parenchyma. The chemokines present in this area in MS lesions are CCL4 [19], CCL5 [30] and CXCL10 [48]. The chemokine receptor expression on infiltrating cells (mostly lymphocytes and monocyte/macrophages) in the perivascular space includes those recognising the chemokines localised to this area, particularly CCR1 [50], CCR2, CCR3, CCR5 [43] and CXCR3 [46].

For infiltrating leukocytes to migrate away from the blood vessel into surrounding parenchyma, it is believed that resident glia such as astrocytes and microglia, express chemokines in response to induction by

Table 2. Chemokine Receptors

CHEMOKINE RECEPTOR	ALTERNATIVE NAMES	CELLS EXPRESSING RECEPTOR*	RECEPTOR LIGAND/S	SYSTEMATIC NAMES FOR RECEPTOR LIGANDS
CXC (α family)				
CXCR1	IL8-RA / Type 1 IL-8R	N, MΦ	GCP-2 IL-8	CXCL6 CXCL8
CXCR2	IL8-RB / Type 2 IL-8R	N, A, Micro	GRO-α GRO-β GRO-γ ENA-78 GCP-2 NAP-2 IL-8	CXCL1 CXCL2 CXCL3 CXCL5 CXCL6 CXCL7 CXCL8
CXCR3	GPR9	T, B, NK	Mig IP-10 I-TAC	CXCL9 CXCL10 CXCL11
CXCR4	HUMSTR / HM89 / LESTR / NPYRL / LCR1 / Fusin	L, EC, Neu, A	SDF-1α/β	CXCL12
CXCR5	BLR-1	B, T (memory)	BCA-1	CXCL13
CXCR6	Bonzo / TYMSTR /STRL33	DC, T	CXCL16	CXCL16
CC (β family)				
CCR1	CC CKR1 / HM145 / MIP-1α/R / MIP-1α/RANTES	M, MΦ, T, G, DC	MIP-1α RANTES MCP-3 HCC-1 MIP-1d / HCC-2 MPIF-1 LD78β Unknown HCC-4	CCL3 CCL5 CCL7 CCL14 CL15 CCL23 CCL3L1 CCL9/10 CCL16
CCR2	CC CKR2A / CC CKR2B / MCP-1RA / MCP-1RB	M, MΦ, L, DC, Ba, NK	MCP-1 MCP-3 MCP-4 HCC-4	CCL2 CCL7 CCL13 CCL16
CCR3	CC CKR3 / CMKBR3 / CKR-3	Th2 cells, Ba, E, mast cells, M, MΦ, Micro	Eotaxin-1 Eotaxin-2 Eotaxin-3 MCP-2 MCP-3 MCP-4 RANTES MIP-1δ / HCC-2 MEC	CCL11 CCL24 CCL26 CCL8 CCL7 CCL13 CCL5 CCL15 CCL28
CCR4	CC CKR4 / K5-5	T, Ba, P, M	TARC MDC	CCL17 CCL22
CCR5	CC CKR5 / CMKBR5 / ChemR13	M, MΦ, memory T cells, DC, Micro, EC	MIP-1α MIP-1β RANTES MCP-2 HCC-1 LD78β	CCL3 CCL4 CCL5 CCL8 CCL14 CCL3L1
CCR6	DRY6 / CKR-L3 / GPR-CY4 / STRL22	Memory T, B, DC	LARC / MIP-3α	CCL20
CCR7	EB1 / BLR2	L, DC, NK	MIP-3β / ELC SLC	CCL19 CCL21
CCR8	ChemR1 / TER-1 / GPR-CY6 / CKRL1	T	I-309	CCL1
CCR9	GPR-9-6	T, thymocytes	TECK	CCL25
CCR10	GPR-2	T, F, EC, DC	CTACK/ALP/IL C MEC	CCL27 CL28

(Table 2). contd.....

CHEMOKINE RECEPTOR	ALTERNATIVE NAMES	CELLS EXPRESSING RECEPTOR*	RECEPTOR LIGAND/S	SYSTEMATIC NAMES FOR RECEPTOR LIGANDS
C (γ family)				
XCR1	GPR5	Under investigation	Lymphotactin SCM-1α SCM-1β	XCL1 XCL2
CX3C (δ family)				
CX3CR1	V28 / CMKBRL1 / GPR13	T, NK, M, Micro	Fractalkine	CX3CL1

*Abbreviations : A = astrocytes, B = B cells, Ba = basophils, DC = dendritic cells, E = eosinophils, EC = endothelial cells, Epi = epithelial cells, F = fibroblasts, G = granulocytes, K = keratinocytes, L = lymphocytes, M = monocytes, Me = mesangial cells, Mega = megakaryocytes, MΦ = macrophages, Micro = microglia, N = neutrophils, Neu = neurones, NK = natural killer cells, Ost = osteosarcoma cells, P = platelets, SM = smooth muscle cells, T = T lymphocytes, Tu = tumour cell lines
The nomenclature for chemokines and their receptors used in this table are based on the IUIS/WHO subcommittee recommendations. (Cytokine **21** (2003) pp. 48-49)

proinflammatory cytokines like TNFα and IFNγ produced by leukocytes in the perivascular space. Expression of CCL2, CCL3 and CXCL10 has been shown in astrocytes and particularly in the foot processes of astrocytes [36,46]. Astrocytes also express chemokine receptors that are specific for the expressed chemokines, CCR2, CCR3, CCR5 [43] and CXCR3 [42]. Microglia express CCL3 [24], CCL4 [19] and CXCL1 chemokines [26] and CCR2, CCR3 [43], CCR5 [46] and CXCR2 receptors [26]. This pattern of expression in resident glia may indicate that these cells play a role in amplification of the inflammatory response.

The CSF reflects the composition of the extracellular fluid in the CNS. The high levels of the T cell chemoattractants CCL2, CCL5 and CXCL10 and the increased expression of CCR5 and CXCR3 indicate that these chemokines are involved in the accumulation of T cells in the CNS [32].

The importance of these chemokines and receptors in the pathogenesis of MS has been supported by findings in EAE. CCL3, CCL4 and CXCL10 are expressed by the vascular endothelium [54], and CCL2, CCL3, CCL4, CCL5 and CXCL10 are expressed by infiltrating leukocytes in the perivascular space and inflammatory foci in EAE [54-58].

Astrocytic expression of chemokines plays a major role in the pathogenesis of EAE. CCL2, CCL3, CCL4 and CXCL10 are all expressed by astrocytes in EAE [54,56,59]. The importance of the receptors for these chemokines in EAE has mainly been shown through chemokine receptor knockout models. If mice are deficient in either CCR1 or CCR2, the incidence of EAE is reduced [60-62]. Furthermore, the importance of CCR1 and CCR5 was highlighted by the finding that blocking CCL3, a ligand for these receptors, prevented clinical signs and mononuclear cell infiltration into the CNS [63]. However, Tran *et al.* [64] showed that CCR5 deficiency does not inhibit the development of EAE. This apparent redundancy of CCR5 in the development of EAE is supported by studies in humans that have a CCR5 deletion mutation. These people do not display any protection against the development of MS [65]. There are few studies in EAE regarding CXCR3 expression; however, Alt and Laschinger *et al.* [66] reported that encephalitogenic T cells showed surface expression of CXCR3 in EAE.

Temporal Expression of Chemokines and Their Receptors in MS and EAE

There are different subtypes of MS, which are recognised based on the temporal features of the disease. These are relapsing–remitting MS (RRMS), secondary progressive MS (SPMS), and primary progressive (PPMS). In RRMS, which is the most common form of MS, patients experience periods of disease exacerbation followed by periods of complete or partial remission. In about 50% of cases of RRMS, the disease eventually follows a secondary progressive course (SPMS). In PPMS, disease is progressive from the onset. Only a small number of studies have investigated whether patterns of chemokine or receptor expression exist that are specific to each subtype of MS. More work has gone into investigating differences between relapse and remission in RRMS and relapsing models of EAE.

PPMS differs from RRMS/SPMS not only in its temporal features but also in the degree of CNS inflammation, with PPMS being characterised by less inflammation than RRMS. It would therefore be expected that the chemokines and chemokine receptors expressed might differ between the different forms of MS. Balashov *et al.* [24] reported that T cells expressing CXCR3 were increased in the blood of patients with RRMS, whereas both CCR5 and CXCR3 positive T cells were increased in the blood of patients with progressive MS. Several papers have compared well defined PPMS with other forms of MS [35,41,67]. Two of these studies found that there was increased expression of CXCL10 in peripheral blood mononuclear cells [67] or serum and CSF [41] in RRMS and SPMS but not in PPMS. In contrast, Martinez-Caceres *et al.* [35] found that CXCL10 was increased in the CSF of some MS patients; however, expression did not correlate with the clinical form of disease.

Several polymorphisms in chemokine receptor genes have been described in different subtypes of MS. One study has investigated whether 2 polymorphisms, one within the CCR5 receptor (δ32) and one in the G-protein β3 subunit (C825T), occur at different frequencies in patients with PPMS compared to RRMS and SPMS [68]. The δ32 polymorphism results in a 32 base pair deletion in the gene, and individuals who are homozygous for this deletion do not produce a functioning CCR5 receptor [69]. This deletion is not protective against development of MS [65]. In contrast, the C825T polymorphism results in enhanced G-protein signalling, with enhanced chemotaxis of human neutrophils as well as enhanced T cell responses and increased immunoglobulin formation [70]. Patients with PPMS showed a trend towards reduced frequency of δ32 CCR5 polymorphism and increased frequency of the C825T polymorphism compared to other MS patients, although the numbers did not reach statistical significance [68].

In patients with RRMS, several chemokines and chemokine receptors have been found to be upregulated on peripheral blood CD4+ cells, in the serum or the CSF, and in the lesion itself during relapses of MS and to return to baseline on clinical recovery. These include: CCR5 expression on peripheral blood mononuclear cells [34] and on T cells in CSF [37]; CXCR3 expression on CD4+ lymphocytes in the peripheral blood [34] in CSF [37,45], and in the lesion [46]; expression of CCL2, CCL3, CCL4, CCL7 and CCL8 within the lesion [19,36]; and CCL5 expression in serum and on the vascular endothelium, perivascular cells and surrounding astrocytes [19,44]. CXCL10 was found to be strongly released intrathecally in RRMS; however, Sindern *et al.* [45] reported that the levels did not vary with magnetic resonance imaging (MRI)-monitored disease activity, whereas Franciotta *et al.* [25] found greater concentrations of CXCL10 in CSF during acute attacks.

It is easier to dissect out temporal activation or downregulation of chemokine genes in EAE models of MS than in MS itself, due to the availability of CNS tissue. Various EAE models exist, which mimic temporally the acute inflammation of a relapse of MS (acute EAE), or long-term relapsing-remitting disease (chronic EAE).

Early studies with acute EAE models identified CCL3 and CCL5 as the chemokines of greatest importance in the induction of EAE [63,71,72]. In support of those early findings, more recent studies have found that CCR1, which is a receptor common to CCL3 and CCL5, is also critical for successful induction of EAE [60,73]. In addition, the CXC chemokines CXCL1 [72] and CXCL10 [73], and the CCR2 receptor [62] are also upregulated during acute EAE. Recently, Kohler *et al.* [74] showed that CCL20 is important in the induction but not in the effector phase of acute EAE.

In chronic EAE the chemokine/chemokine receptor combination most often reported to be associated with relapses of disease is CCL2/CCR2 [63,75,76]. CCL20, produced by intraparenchymal astrocyte-like cells, has also been reported to correlate closely with disease relapses [77]. In contrast, CCL3 and CCL5 expression do not appear to fluctuate with relapses and remissions of EAE [76].

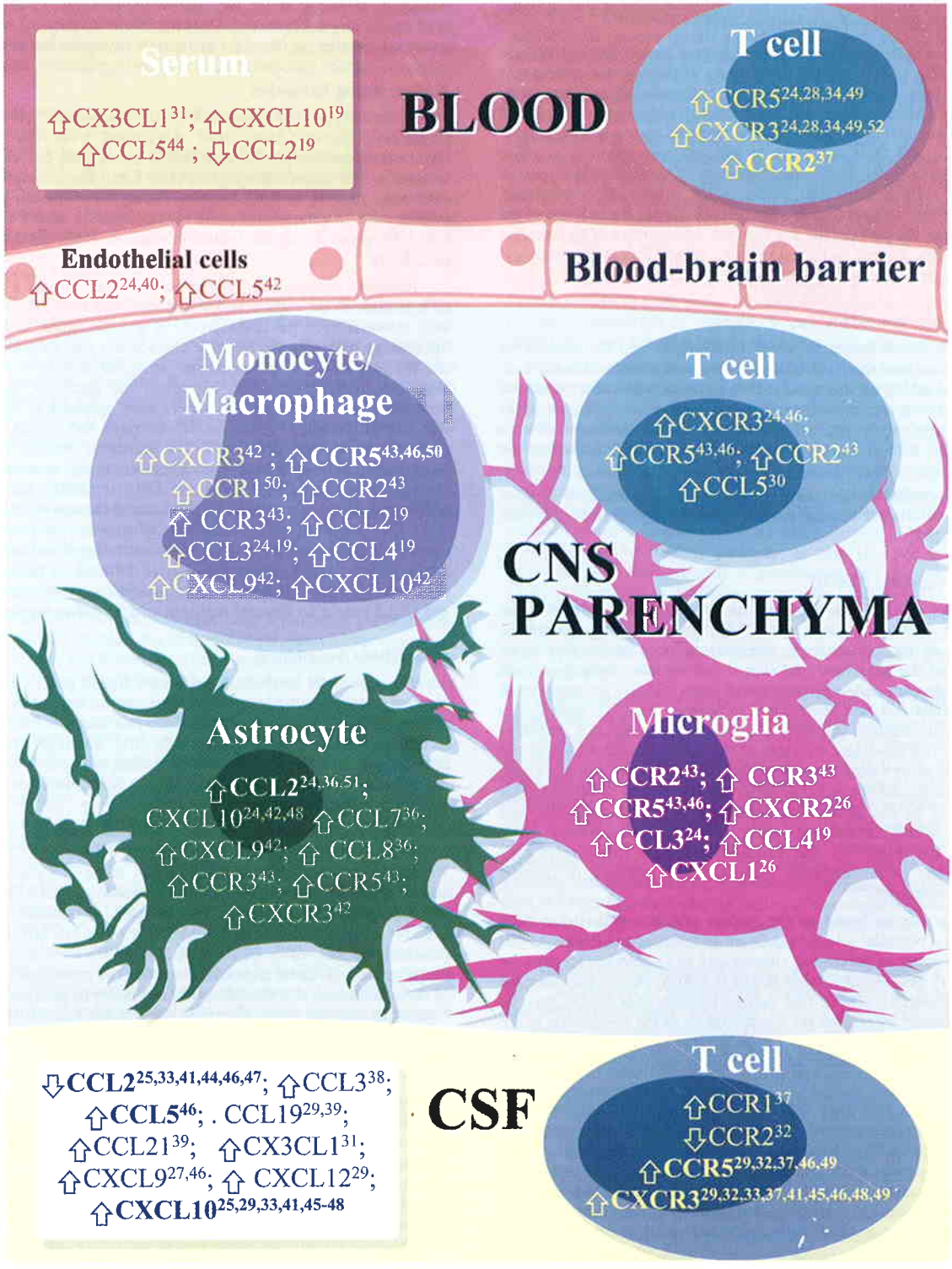

Fig. (2). Spatial expression of commonly expressed chemokines and chemokine receptors in MS.

THE CHEMOKINE SYSTEM AS A TARGET FOR THERAPY IN MULTIPLE SCLEROSIS

Current therapeutic agents for MS are generally non-specific anti-inflammatory immunomodulatory agents (see review by Pender and Wolfe [78]). Among available therapies, IFNβ and glatiramer acetate have a modest effect on reducing relapses and slowing the accumulation of disability in relapsing-remitting MS. IFNβ is of doubtful efficacy in secondary progressive MS and appears to aggravate primary progressive MS, possibly by increasing antibody-mediated CNS damage through inhibition of B-cell apoptosis [78]. Mitoxantrone reduces relapses and slows disability progression in relapsing-remitting and secondary progressive MS, but its use is limited by the risk of cardiomyopathy. There is currently no effective treatment for primary progressive MS.

Some of the currently used therapies effect chemokine/receptor expression. CCR5 and CXCR3 [49,79] have been found to be downregulated on $CD4^+$ and $CD8^+$ T cells in MS patients treated with

IFNβ. In addition, levels of CCL2 in CSF were found to increase significantly in patients treated with methylprednisolone and to correlate negatively with measures of inflammation [47].

Chemokines and their receptors are obvious targets for intervention strategies for MS, as they are the components of the immune system that orchestrate cellular migration. Inhibiting the inflammatory response early in the course of MS should reduce the frequency and severity of relapses and slow the progression of disability. The temporal and spatial expression of chemokines and their receptors in MS can be exploited to tailor therapies to specifically block the migration of particular subtypes of inflammatory cells at particular points in the inflammatory response, potentially without nonspecifically affecting the normal functioning of the immune system. The past decade has seen the development of therapeutics for the chemokine system go through several stages. Potential targets and strategies for therapeutic intervention in MS are outlined below.

1. Production of Chemokines

One of the initial stages at which intervention into the chemokine system can occur is at the level of chemokine production within the cell. One method of inhibiting this process is to interfere with the translational events using antisense technology. Precise nucleic acids sequences or antisense oligonucleotides are designed to hybridise to specific mRNAs in order to interfere with the normal translation of that mRNA and reduce the expression of the coded protein.

Natural phosphodiester antisense oligonucleotides directed against CXCL10 reduce clinical disease activity when infused intrathecally into rats with EAE [80].

2. Glycosaminoglycans (GAGs)

In order for potent chemokine gradients to be established within the CNS to lure leukocytes into the CNS from the low chemokine concentration in the bloodstream, chemokines need to be able to be immobilised on the abluminal endothelial cell surface. Specialised cell surface proteoglycans called glycosaminoglycans (GAGs) are believed to be able to interact and bind chemokines to achieve this [81]. Such GAGs include heparin, heparin sulphate, chondroitin sulphate and dermatan sulphate [82]. The molecular structure of the GAGs is determined by the location and type of vascular endothelium on which they are expressed, and, on this basis, chemokines will differentially bind to different GAGs [83]. This may be the factor that determines which chemokines, and subsequently which leukocytes, are present at inflammatory sites. Furthermore, the selectivity that exists within these interactions may provide a means of manipulating the chemokine network in specific inflammatory responses.

GAGs can also be found on the surface of leukocytes. It has been suggested that a potential role for GAGs on endothelial and leukocyte cell surfaces is to assist in presenting chemokines to G protein receptors on target cells [84]. Interestingly, when bound to GAGs, chemokines have the ability to oligomerise. These two factors indicate that GAGs may be important in firstly, increasing the concentration of the chemokine in the immediate area of target receptors (i.e. on the leukocyte itself) to increase binding efficacy and secondly, in establishing more potent chemokine gradients [85].

The important role that GAGs play in inflammation has been demonstrated by experiments showing that the affinity of cells expressing CXCR1, CCR1 or CCR2 to bind RANTES, MCP-1, Interleukin (IL)-8 and MIP-1α was significantly decreased after treatment of cells with glycosidase to remove GAGs [85]. Furthermore, it has been recently reported that when the GAG binding sites on CCL1, CCL4 and CCL5 are mutated, these chemokines lose their ability to form oligomers and recruit leukocytes *in vivo* [86].

GAGs also exist in a soluble form. Unlike immobilised GAGs, soluble GAGs appear to play an inhibitory role in the chemokine system. Soluble GAGs have the same binding capabilities as immobilised GAGs; however, when a chemokine binds to a soluble GAG, it is unable to subsequently bind with its receptor, therefore blocking the biological activity of that chemokine [84].

There is some evidence for a beneficial effect on EAE of treatment with soluble GAGs. Treatment with heparin, but not chondroitin-4-sulphate, inhibits the development of EAE in mice and rats [87,88]. Furthermore, a synthetic heparin-mimicking and low anticoagulant compound, RG-13577, also ameliorated the clinical signs of acute EAE in mice [89]. These reports have considered the action of the GAGs to be at the level of inhibiting heparinase activity, which has been implicated in BBB breakdown; however, an alternate explanation is that the GAGs are binding to chemokines and blocking their activity. GAGs provide a potentially interesting target for therapeutic manipulation in MS.

3. Neutralizing Antibodies

Several types of chemokine- or chemokine receptor-specific therapeutic agents have been developed over the past decade. Neutralising antibodies have provided proof that the chemokines are integral to the inflammatory process in EAE. For example, neutralizing antibodies specific for CCL2, given during the remission phase of EAE, inhibit further relapses of EAE [75], whereas anti-CCL3 and anti-CXCL10, given during the induction phase of EAE, inhibit acute disease [63,73,90].

Antibody neutralisation of chemokines does present a potential level of regulation in the inflammatory response, and such experiments provide vital information in the development of drug therapies, including specific information regarding the role that chemokines play in neuroinflammation and the side effects of removing the biological activity of that chemokine from the immune response. However, the potential use of antibody neutralisation of chemokines as a therapeutic approach in MS is remote, as the cost of antibody production is excessive and would be difficult to support over an extended period. An alternative method of induction of neutralising antibodies has been explored using vaccination with CC chemokine naked DNA vaccines. DNA vaccines cloned from the polymerase chain reaction (PCR) products of chemokine mRNA extracted from the brains of rats with EAE effectively protected against the development of acute EAE [91]. Administration of an anti-CCL2 DNA vaccination during relapsing EAE reduced severity of disease [92]. Youseff *et al.* [92]also showed that naked DNA vaccines to CCL2 and CCL3 induce an immune response which confers resistance to EAE.

4. Modified Chemokines

The successful interaction between a ligand and its receptor can be manipulated so that the target cell does not become activated. Modified chemokines have been developed to interfere with this interaction in order to inhibit signalling in the target cell. The amino (N) terminus of the chemokine appears to be essential in binding with the G protein receptor. It has been shown that by modifying this N terminal portion, the target chemokine receptor can be antagonised.

In studying the function of the N-terminal region of CCL2, Gong and Clark-Lewis [93] discovered that a truncated form of CCL2, residues 9-76, bound specifically to its receptor but, instead of eliciting activation, was a potent desensitiser of the Ca^{2+} response to CCL2. In later studies, the same group showed that another truncated chemokine, CCL5 residues 9-68, was promiscuous in its ability to inhibit cell migration and inflammation induced by CCL2, CCL5, or CCL7, even though the receptor usage by these different chemokines varies. The promiscuity in the binding of these analogues and the successful inhibition of activation indicate that N-terminal residues partly determine the receptor specificity of CCL5, and deletions within this region permit binding to multiple chemokine receptors. The findings suggest that it may be feasible to design high affinity multi-specific CC chemokine antagonists for therapeutic intervention [94].

One of the most extensively studied modified chemokines is Met-RANTES, an N-terminally modified chemokine (CCL5) that has been found to have antagonistic activity towards two rodent chemokine receptors CCR1 and CCR5 [95,96]. When administered at disease onset, Met-RANTES has been shown to significantly ameliorate neurological disability at the endpoint of chronic EAE, although it had no effect on treatment of acute EAE [97]. The mechanism of action of Met-RANTES in chronic EAE is not certain, as it does not reduce cellular infiltration into the CNS or inhibit upregulation of CCR1 or CCR5, suggesting that is has no effect on the activation state of leukocytes. It has been suggested that it may reduce the microglial/macrophage-mediated destruction of axons [97].

Other modified chemokines that have been tested *in vivo* are the CXCR2 antagonist GROα(8-73), a truncated form of CXCL1 that can prevent neutrophil recruitment in acute inflammation *in vivo* in mice [98], and a truncated form (residues 4-73) of CXCL11, the most potent ligand for CXCR3, that can compete for the binding of native CXCL11 to CXCR3-bearing cells, inhibiting migration and Ca^{2+} mobilisation [99].

Modified chemokines that can antagonise chemokine activity also occur naturally through proteolytic processing of chemokines. Matrix metalloproteinases (MMPs) are thought to play a role in this process. It has

been shown that CC chemokines treated *in vitro* with MMPs produce truncated forms of the chemokines that can act as potent antagonists of their receptors *in vitro* [100]. MMPs are thus thought to play a role in the resolution of inflammation both by degrading pro-inflammatory chemokines and generating truncated chemokines that inhibit chemokine signalling.

Despite the successes using modified chemokines in animal models, and the recent demonstration that a variant of CCL3, BB100010, was found to be well tolerated and achieve satisfactory levels in plasma in human trials [101], the development of modified chemokines as therapeutics is not progressing very rapidly. This is probably due to the increased interest by pharmaceutical companies in the development of small molecule antagonists.

5. Small Molecule Antagonists

The seven-transmembrane G protein-coupled receptors have been targeted by pharmaceutical companies for many years because of their efficacy as targets for small molecule drugs. Because chemokine receptors fall into this category of receptors, small molecule antagonists have become the focus of interest for development of specific and potent therapies to modulate the chemokine system. Recombinant receptors are used to screen thousands of molecules in an effort to find molecules of potential therapeutic use, and then these molecules are tested *in vivo*.

The first potent and selective non-peptide inhibitor to appear in the literature was SB 225002, which affects CXCR2 binding and downstream calcium flux, and blocks neutrophil chemotaxis and adhesion *in vitro* [102,103], and IL-8 mediated neutrophil trafficking *in vivo* [104]. Since then, many small molecule antagonists have been investigated. Of those, several are being considered as potential therapeutic agents for MS, particularly those targeting CCR1, CXCR3 and CCR5.

A number of CCR1 antagonists have been reported in the literature, including those from Takeda Chemical Industries, Banyu Pharmaceutical Co., Merck and Co., and Pfizer Inc. (See Horuk and Ng [105] and Saeki and Naya [106] for comprehensive reviews of these chemokine receptor antagonists). The CCR1 antagonist that has been the main focus of attention is BX471, a potent and selective, orally available CCR1 antagonist from Berlex [107], which is reviewed in Elices [108]. BX471 is a 4-hydroxypiperidine analogue that can displace CCR1 ligands CCL3, CCL5 and CCL7 with high affinity, inhibit calcium mobilisation, and increase extracellular acidification, CD11b expression, and leukocyte migration [107]. In a rat EAE model, BX471 was shown to reduce the clinical severity of disease in a dose-dependent manner, and BX471 is now in Phase 1 clinical trials for MS [109].

The use of CCR5 antagonists has been mainly confined to HIV research, and research into CXCR3 antagonists is only just a beginning. TAK-779 antagonises the binding of CCL5 to CCR5-expressing cells and blocks CCR5-mediated signalling. In addition, TAK-779 blocks ligand binding and cell migration effects of CXCR3 [110]. By blocking both receptors, TAK-779 effectively blocks the chemotaxis of $CCR5^+/CXCR3^+$ cells, which are found to be predominantly Th1 cells. It thus has potential use for MS. One problem with TAK-779, however, is that it is a quaternary ammonium salt, which limits its use due to poor oral absorption and rapid elimination [105].

Many challenges face pharmaceutical companies in the development of small molecule inhibitors of chemokine receptors. The most important is that the chemokine receptors are closely related to other G protein-coupled, seven transmembrane receptors, thus increasing the possibility that small molecule antagonists intended for chemokine receptors could cross react with other receptors. For example, BX471 binds to several biogenic amine neurotransmitter receptors, including dopamine and muscarinic receptors, albeit at significantly higher concentrations (>50 times) than to CCR1 [111]. Other small molecule antagonists that show cross reactivity to neurotransmitter receptors are the piperazine-based CCR5 inhibitors from Schering Plough that cross react with muscarinic receptors [112], the CCR2 spiropiperidine series from Roche which cross react with serotonin receptor-1a as well as α_1 adrenergic receptors [113], and another CCR2 inhibitor from Smith Kline Beecham that also cross reacts with serotonin receptors [114]. Species selectivity is also a problem in testing CCR antagonists, as many of the small molecule antagonists have poor affinity for nonhuman receptors. BX471, for example, binds with high affinity to human receptors but with low affinity to rodent CCR1 [107]. This makes it difficult not only to assess the efficacy of human-specific agents using animal models, but also to determine therapeutic doses for clinical trials.

6. The Chemokine Receptor: Expression-Downregulation and Decoys

When a chemokine receptor interacts with its ligand, it not only causes activation of the cell via the signal transduction pathway but also leads to downregulation of the receptor on the cell surface [115]. This feedback mechanism is thought to serve several purposes: to attenuate receptor-mediated responses, to increase the sensitivity of the cells to small changes in chemokine gradient, to assist in transendothelial migration, or to prevent accumulation of chemokines at the inflammatory site. Receptor down-modulation induced by chemokines may be an important mechanism for turning off the activation signal in inflammatory cells in MS, and may be one of the mechanisms leading to periods of remission from disease. Recently, Putheti *et al.* [116] have shown that MS patients with high active lesion load in the CNS have decreased levels of CXCR3 on blood CD4+ T cells compared to patients with fewer lesions, suggesting that internalisation of CXCR3 is occurring due to the release of its chemokine ligand (CXCL10) from active MS lesions. Understanding how chemokine receptor expression is modulated and regulated could lead to the identification of potential targets for therapeutic intervention.

Several papers have investigated the mechanism of chemokine receptor down-modulation. Internalisation of chemokine receptors following ligand binding could potentially occur *via* arrestin-mediated movement of the receptor into clathrin-coated pits, or *via* microdomains in the plasma membrane known as caveolae, which play a role in endocytosis and transcytosis. Using inhibitors, Mueller *et al.* [117] showed that both pathways are used in CCR5 internalisation, with different chemokine ligands interacting with CCR5 mediating different patterns of cellular responses. In addition, CCR5 internalisation is thought to be closely linked to the proteasome pathway, as this receptor is constitutively associated with the ζ subunit of proteasome, and the downregulation of CCR5 is attenuated by inhibiting proteasome function [118]. The processes involved in the recycling of the CCR5 receptor are postulated to involve transport of the receptors into endosomes, where they are dephosphorylated and resensitised before being presented back on the cell surface [117].

Internalisation of CXCR2 is mediated by arrestin/dynamin mechanisms, is kinase-dependent, and involves phosphorylation sites in the cytoplasmic tail of the receptor [119,120]. In phagocytosing human neutrophils, however, downregulation of CXCR2 is due to surface cleavage of the receptors by MMPs, not to internalisation [120-122]. Degradation of the receptor significantly reduces the ability of neutrophils to respond to CXC chemokines after they have begun phagocytosis [121]. Sauty *et al.* [123] have shown that the signal transduction pathway mediating agonist-induced CXCR3 internalisation is distinct from the pathway that mediates CXCR3-induced chemotaxis. Furthermore, not all CXCR3 ligands induce internalisation of the receptor to the same degree. This suggests that it may be possible to target specific ligand/receptor interactions important in a particular disease.

Maintenance of receptors on the cell surface, but in a non-signalling form, can also act to modulate the chemokine system. It has been shown that anti-inflammatory cytokines such as IL-10 can act to uncouple chemokine receptors from the G-protein signalling machinery, thereby inhibiting cell activation and migration. Essentially, these receptors act as scavengers to inhibit biological activity and accumulation of chemokines at the inflammatory site [124], and are called decoy receptors [125]. Functional decoy receptors for CCR1, CCR2 and CCR5 have been reported in monocytes and dendritic cells. Two naturally occurring cell-surface receptors have also been reported to act as decoys by binding chemokines but not providing signalling activity: DARC (Duffy antigen receptor for chemokines) and D6. DARC is highly promiscuous and is expressed on a variety of erythroid and non-erythroid cells including brain cells [125]. D6 binding is restricted to CC chemokines [126] and it has been shown that although CC chemokine ligands are bound to the decoy receptor, there is no cellular activation, confirming its role as a scavenger for chemokines [127]. The possibility of modulating expression of these decoy molecules to change how chemokines act in a particular anatomical location remains to be explored.

7. Signal Transduction

Chemokine production is regulated by transcriptional events induced by a variety of stimuli, such as IFNβ-, IFNγ-, IL1β-, or TNF- induced activation of nuclear factor κB (NFκB), activator protein 1 (AP-1) and Jak/Stat signalling cascades [128-130]. The signal transduction pathways triggered by receptor-ligand interactions in chemokine signalling are complex and thus provide a large number of candidate molecules for manipulation of the process. A number of enzymes involved in these

events are under investigation, in particular phosphoinositide 3-kinase γ (PI3Kγ), as it has been shown to play a crucial role in macrophage accumulation in inflammation [131,132], and members of the Rho family, which are involved in cell motility-associated events [133]. This field of research is in its infancy; however, it holds the potential for specifically manipulating the chemokine receptor signalling activity responsible for cellular responses in inflammation.

8. Viral Mimicry

It has been well documented that viruses, particularly the large DNA viruses such as herpesvirus, poxvirus and retroviruses, have the ability to manufacture molecules that "mimic" host chemokines and chemokine receptors in order to facilitate viral invasion, replication and survival within the host cell. There are three types of viral chemokine mimics that can manipulate the chemokine system: chemokine homologues, which act as chemokine receptor antagonists; chemokine receptor homologues, which act as membrane-expressed chemokine scavengers; and non-chemokine or chemokine-related structures, which act as secreted chemokine scavengers. Of the thirteen known chemokine homologues, six are CC chemokines, and seven are CXC chemokines [134].

There is the potential for using these virally encoded chemokine and chemokine-binding proteins as therapeutic targets in diseases like MS. For example, the virally encoded CXCL2-like receptor antagonist (vMIP-2) produced by human herpesvirus 8 (HHV8) has the ability to antagonise six chemokine receptors [135]. In addition, viruses naturally produce chemokines that bias the immune response to a virus-friendly Th2 type of response, as is the case with vMIP-2 [136]. Direct inhibition of chemokine activity could be achieved using poxviruses, that can encode chemokine-binding proteins that block the GAG-binding epitope on chemokines and the receptor-binding epitopes on many CC chemokines [137]. Another chemokine-binding protein, encoded by a murine herpesvirus, has been identified that can bind chemokines across all four families and inhibit their activity [138].

However, there are inherent problems associated with using viruses [139]. These virally encoded proteins are designed to work within the immediate area of the virus. Thus, for MS, the virus would need to be delivered into the CNS. Intrathecal delivery, while possible, is not the method of choice for on-going therapies, particularly in an autoimmune disease such as MS, in which there is an underlying dysregulation of immune responses.

9. Polarity of Response

Chemokines differentially recruit Th1 and Th2 effector cells to sites of inflammation [140]. In chronic EAE, there is increased expression of CCR1 and CCR5, which are associated with Th1 helper cells, but no increased expression of CCR3 and CCR4, which are associated with Th2 cells [55]. Furthermore, it has been shown that clinical EAE can be downregulated by passive transfer of regulatory T cells with a Th2 phenotype [141], and that immunisation with altered peptide ligands, which induced preferential downregulation of Th1 cells and skewing the response towards a Th2 polarity, led to expression of CCR3 and CCR4 in protected mice [54]. Attempts to induce such a polarisation shift by using chemokines are under investigation. One possible approach was investigated by Wildbaum *et al.* [142], who showed that injecting plasmid DNA encoding self-CXCL10 drove the polarisation of T cells towards a Th2 phenotype and conferred resistance to EAE on the recipients.

In developing these types of approaches for therapeutic intervention, interpretation of results must be approached carefully, as there are differences in the expression of chemokine receptors *in vitro* vs *in vivo,* which can have profound effects on disease. For example, CCR8, the receptor for CCL1, has been detected *in vitro* on Th2 cells [143]; however, CCR8 was not expressed on T cells *in vivo* but was associated with macrophages and activated microglia in MS lesions and directly correlated with demyelinating activity [144]. Furthermore, it has been shown that Th2 responses are not always protective and in some experimental systems are detrimental. Lafaille *et al.* [145] showed that Th2 polarised T cells can induce EAE, and Genain *et al.* [146] reported high levels of Th2 cytokines and anti-myelin antibodies associated with worsening of EAE in marmoset monkeys after tolerisation to myelin oligodendrocyte protein (MOG). A Th2 response may also be harmful in MS by increasing the level of pathogenic autoantibodies.

CONSIDERATIONS FOR THERAPEUTIC USE

Owing to the complex nature of MS, it is sometimes difficult to determine which of the immune responses occurring during the course of disease are beneficial or regulatory, and which are not. There may be a role for inflammatory chemokines in CNS tissue protection [147] and repair [148] as well as in disease, so blocking all inflammation may not be advantageous. For example, Weber and colleagues [148] have shown that CCL2 and CCR2 expression by endothelial cells can have profound effects on endothelial wound repair, which could be useful in the repair of blood–brain barrier injury. In addition, because of the important role of the chemokine system in homeostasis of the immune system, therapeutics need to differentiate the inducible messengers of inflammation that are upregulated during disease from the homeostatic controllers and coordinators.

When considering therapeutic agents for treatment of MS, the drug should preferably have specificity for the disease process and have no toxicity; it should have pharmacokinetic and pharmacodynamic properties that will enable it to enter the CNS and act for a sufficient length of time to exert a beneficial effect; and it should be reversible if required.

CONCLUSIONS

A large body of literature exists regarding chemokines and chemokine receptors in MS and the animal model EAE, and how these molecules contribute to the disease process. Many potential candidates for therapeutic intervention targeting chemokines and their receptors have been considered, and some of these show potential for the treatment of MS. In developing therapies for MS, it is important to understand the spatial and temporal characteristics of the disease in order to develop appropriate interventions, and remember that some processes, which are pathogenic in one setting are beneficial in another. Extrapolating findings in EAE should be done with caution. Although studies in EAE provide valuable insights into neuroinflammation and a means of investigating potential therapies, it must be remembered that the effects of therapies in EAE are the product of a defined set of circumstances in a controlled and well understood experimental system and might not be applicable to the unpredictable and poorly understood disease process of MS.

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